Biochemical Modulation of 5-fluorouracil by uridine and leucovorin

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Chapter 10

Summarising Discussion

The fight against cancer is based on several elements: prevention, early diagnosis, surgical removal, radiation treatment and drug therapy. The goal is to increase cure and to reduce the consequences of treatment. Progress has been observed in all these aspects, even if to a different extent in the different tumour types.

Concerning colorectal cancer, screening with colonoscopy after the age of fifty represents a very effective technique, but it is not widely accepted by the general population. This is one of the reasons why this remains a very common cancer even if at present it is often diagnosed at a relatively early stage. In this situation it is possible to cure the majority of patients by the surgical removal of the tumour. When cancer has spread to the regional lymph nodes, however, survival can be improved by the postoperative administration of chemotherapy (adjuvant treatment). Even when colorectal cancer has spread to the liver or to the lungs, metastases can be resected with excellent results. In many metastatic patients, however, chemotherapy is the only available treatment.

For many years the only drug with a consistent activity against colorectal cancer was 5-Fluorouracil (FU) and this is the reason why this drug has been extensively studied in this disease. It was necessary to use FU at its best in order to improve the meagre results. Today other conventional drugs have been added to the medical treatment of colorectal cancer (Irinotecan, Oxaliplatin) and monoclonal antibodies (Cetuximab, Panitumumab, Bevacizumab) but FU still represents a necessary element of treatment.

Unfortunately the list of ineffective drugs that have been tested against colorectal cancer is very long: not only other fluoropyrimidines, but also anticancer drugs that are very effective against other cancers and, more recently, inhibitors of tyrosine kinases that act on the epidermal growth factor receptor pathway and/or on angiogenesis.

The first successful method to improve FU activity in colorectal cancer was through “modulation”. This term indicates the possibility of influencing the activity of FU by providing cofactors that would preferentially direct its activity against a specific target. As discussed in chapter 2 several agents have been proposed as “modulators” of FU: a general principle is however that these agents should have no or limited anticancer activity by themselves. For methotrexate a direct modulation is plausible, even if this role might be played by the administration of folinic acid that was given as a rescue from Methotrexate toxicity.

Even if chapter 2 was written many years ago, no new agents have appeared as modulators of intravenous FU since then, and only folinic acid is still widely used today in clinical practice. It is interesting to note that Cisplatin was proposed as a modulator of FU activity: today this drug is only very rarely used in colorectal cancer, while Oxaliplatin is commonly associated with FU, but with no claim of a modulating role.

5-Fluorouracil is an interesting drug not only due to its complex metabolism and to the presence of multiple sites of antiproliferative action in the cell, but also because it is rapidly cleared from plasma. The half life of the parent compound is 10-15 minutes. This makes it an ideal candidate for the prolonged administration through intravenous infusion. The widespread clinical use of this schedule, however, was only possible when small and

convenient infusion pumps became available: prolonged FU infusion (lasting several weeks) was compared with standard i.v. push. Clinical trials in patients gave disappointing results: even if the response rates were higher for the prolonged infusions, survival was not improved. The main difference, however, consisted in a lower haematological toxicity of infused FU: this made it very suitable for combination with myelotoxic agents and/or with radiotherapy. It was even suggested that bolus and infused FU were almost two different drugs and the two modalities were combined in protocols that had different fortunes.

The prolonged infusion of FU in patients was technically possible much earlier than it was in experimental animals. We were among the first that studied the possibility of reproducing the very long (21 days) infusion of FU in mice using subcutaneously implanted pellets. We demonstrated that the drug was actually delivered during the entire period and that plasma levels were in the micromolar range. Interestingly, we showed that also in the mouse FU concentrations vary with a circadian rhythm: this had already been seen in humans receiving FU by continuous infusion, and is due to the circadian variation in the activity of DPD, the main enzyme in FU catabolism.

We also studied the possibility of using folinic acid as a modulating agent of infused FU. Similarly to what was demonstrated in humans, this combination results in increased gastrointestinal toxicity and does not improve the antitumour activity of FU infusion. This is consistent with the observation that folinic acid does not increase TS inhibition, but rather prolongs FdUMP binding to TS: this effect is not relevant when FdUMP concentrations in tissues are constantly maintained thanks to the prolonged administration of FU.

When FU infusion was compared with bolus treatment in mice implanted with different tumour types results again reproduced the clinical observations: tumour volume was more effectively decreased by infusion, but survival was not different. After a few days in fact tumours started to grow again at a rate similar to that of untreated controls. Biochemical analysis of tumour tissues showed that TS activity, that was reduced during the first days of treatment, showed an impressive rebound after 7-10 days of FU infusion.

We continued to study the pharmacological aspects of bolus FU in humans: the standard dose had already been extensively studied, but few data existed concerning the administration of higher drug doses. We therefore analysed data obtained from patients enrolled in a trial of escalating doses of FU. We showed that the main pharmacological parameter, AUC, was correlated with peak plasma concentration and that it was possible to accurately predict plasma AUC using the concentration measured at 30 minutes after bolus FU injection.

In this trial we also observed a patient who had severe unexpected toxicity even at very low FU doses: this patient was later shown to have an eightfold reduction in the activity of DPD, the rate-limiting enzyme in FU catabolism. His AUC, and the danger of significant toxicity, could also be accurately predicted by FU concentration at 30 minutes.

Fluorouracil is an antimetabolite that inhibits cell proliferation by interfering with the use of the physiological counterparts: Uridine and its derivatives. In the case of other antimetabolites, it has been demonstrated that

it is possible to increase the tolerated drug dose by providing high concentrations of the rescuing physiological metabolite. In the case of methotrexate it is possible to administer very high doses with tolerable toxicity when folinic acid is given starting 6 hours after methotrexate infusion. The high concentrations of methotrexate that can be safely reached with this protocol may be able to overcome cell resistance linked to poor trans-membrane transport.

A similar strategy has been proposed for fluorouracil by using Uridine as a rescuing agent. A further advantage was that the administration of high doses of Uridine in humans was already used to treat a specific metabolic alteration.

In vitro studies on uridine rescue were very promising, but the administration of pharmacological doses of uridine to experimental animals resulted in fever or hypothermia and diarrhoea. A similar effect was seen in humans: the doses of uridine needed to rescue normal tissues from FU toxicity, in fact, were very high, and resulted in intolerable toxicity.

Considering the interesting in vitro results obtained with uridine, but the difficulties caused by its administration humans, the next step was to identify a form of "prodrug" of Uridine that might be administered with lower side effects and increase Uridine concentration in plasma and in normal tissues. We focussed on Uridine diphosphoglucose.

This molecule is used in several biochemical reactions as a glucose donor, for example in the biosynthesis of glycogen in the liver. We studied the effect of UDPG rescue after high-dose FU in mice: UDPG was administered in three doses starting two hours after FU bolus. We showed that FU toxicity in normal tissues was reduced and that it was possible to increase the MTD of FU by 50% (from 100 to 150 mg/kg/week) and that this treatment was more effective than the standard dose against three murine colon tumours (Colon 26, Colon 26-10 and Colon 38). In subsequent experiments we demonstrated that UDPG rescue, at the schedule used in our experiments, did not interfere with the antitumour activity of FU: when standard FU dose (100 mg/kg/week) was administered with or without UDPG rescue, in fact, the antitumour activity did not change.

The next step was to investigate the biochemical explanation of the rescuing activity of UDPG from FU toxicity that did not affect the antitumour activity. The high-dose FU followed by UDPG rescue resulted in prolonged inhibition of TS activity, and UDPG allowed a faster clearance of FUTP from cellular RNA. This element is a further demonstration that in this tumour model the antitumour activity of FU is due to its interference with TS activity, while FUTP incorporation into RNA is mainly responsible for the toxic side effects of treatment.

Fluorouracil is being used since 50 years, and its role in the treatment of many tumour types remains pivotal. New drugs are being combined with it, but it has never been completely replaced. Many aspects of its pharmacology are not yet fully described, and its complex metabolism proved to be an important element in order to improve its antitumour activity. Several drugs have been combined with 5FU in the treatment of colorectal cancer: at present the most widely used schemes are FOLFIRI (with Irinotecan) and FOLFOX (with

Oxaliplatin). Also these polychemotherapy schemes are being improved, and we now use a modified version that is indicated as mFOLFOX-6. An improvement in 5FU dosing should be possible if techniques such as Therapeutic Drug Monitoring are implemented. This is also valid for the oral administration of molecules that act as a prodrug for 5FU: Capecitabine, UFT and S-1. These compounds mimick the prolonged infusion of 5FU since they provide a constant exposure of tissues to the active metabolites but do not require the disadvantages of pumps and of a permanent venous access.
Last but not least “modern” therapies based on the use of (Tyrosine Kinase inhibitors, monoclonal antibodies) still require the presence of 5FU although the optimal schemes of administration still need to be determined.